

A simple l.c. method for the direct analysis of sugar acids and their lactones

Uronic and aldonic acids are often quantitatively determined by anion-exchange chromatographic methods¹⁻⁴. These provide good resolution and selectivity, but usually require much (1-6 h) analysis time. High-performance anion-exchange chromatography^{5,6} allows more-rapid analysis of such compounds, but commercially available columns are prone to rapid degeneration and subsequent loss of resolution. Alternatively, carbohydrate-derived acids may be analyzed by gas-liquid chromatography (g.l.c.), but only after they have been converted into suitably volatile derivatives⁷⁻¹⁰. Correspondingly, aldono- and urono-lactones may be either saponified, and then analyzed as free acids by anion-exchange methods, or converted into volatile derivatives for g.l.c. Occasionally during the course of kinetic or thermodynamic studies, analyses of synthetic reactions, or natural-product investigations, it is of value to be able to analyze directly and simultaneously (without derivatization or interconversions) the various free acids and lactones that may be present in a given mixture. None of the previously mentioned methods, however, are capable of providing this information directly in one chromatographic run.

We have recently completed a detailed investigation¹¹ of the chromatography of a variety of aldonic and uronic acids and their lactones on high-performance liquid chromatography (l.c.) columns packed with polystyrene divinylbenzene-type, strong cation-exchange resins that are eluted with a mobile phase of dilute mineral acid (pH 2). This chromatographic method, which has been called ion-exclusion-partition chromatography¹², is actually gel-permeation chromatography on cation-exchange resins, a process developed by Wheatman and Bauman^{13,14}, later modified by several investigators^{12,15-17}, and recently used for the analysis of selected carbohydrate-derived acids and lactones¹⁸⁻²⁰.

In our study¹¹, we found that, under the appropriate mobile- and stationary-phase conditions (pH and temperature are critical variables), many sugar acids and



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lactones give one sharp, readily quantifiable peak, and furthermore, they showed little tendency either to hydrolyze or lactonize during the chromatographic run, providing that the correct conditions were maintained.

In this system, compounds are separated by a combination of size- and ion-exclusion modes. For compounds of similar molecular weight, ion exclusion becomes an important separation mechanism¹⁷. A mixture of a sugar acid and its lactone is readily separated because the acid ($pK_a \cong 2-4$), which is partially ionized under the chromatographic conditions, will be excluded (by Donnan effects¹⁴) from the internal pore volume of the packing gel and is eluted near the column's interstitial volume. The neutral lactone may partition into the internal volume of the gel and is therefore eluted at a later time, permitting the separation of the acid-lactone pair. A detailed description of the separation mechanisms will be given elsewhere¹¹.

While there are many instances wherein this chromatographic procedure may be useful, we report here on three selected examples: monitoring the extent of saponification of a glycurono-6,3-lactone, determining the equilibrium concentrations of a uronic acid-lactone pair under specific reaction conditions, and analyzing the conversion of an aldonic acid into its various lactone forms.

An example of the use of this method for monitoring the saponification of an aldono-lactone is shown in Fig. 1. Pure mannuronic acid is not readily available and

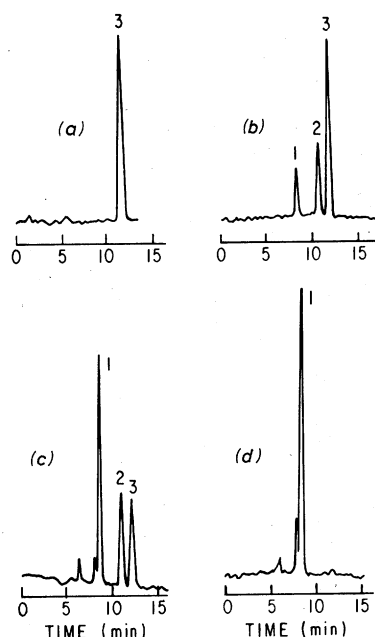


Fig. 1. Saponification of D-mannofuranurono-6,3-lactone (Peak 3), to yield D-mannuronic acid (Peak 1). Figs. *a-d* represent sequential stages in the addition of the equivalent amount of M sodium hydroxide solution: *a*, 0% NaOH added; *b*, 15%; *c*, 75%; *d*, 100%. HPX-87-H⁺ column at 25°, eluted with 5mM H₂SO₄ at 0.6 mL/min. Injection size: 20 μ L. U.v. detection at 220 nm, at 0.2 AUFS (except for *a*: 0.1 AUFS). Initial lactone concentration was 5 mg/mL.

must be prepared by base-catalyzed hydrolysis of D-mannofuranurono-6,3-lactone. The pure lactone (Peak 3 in Fig. 1) was converted into the sodium salt of the free acid (Peak 1) by the addition of M sodium hydroxide. The progress of the reaction was monitored by l.c. (Fig. 1a-d). In addition to revealing the point of complete conversion, the chromatographic procedure also disclosed the presence of an unknown but significant intermediate, Peak 2, and other trace contaminants (minor peaks in Fig. 1d) in the final preparation. No derivatization nor sample cleanup was required for these rapid (12-min) analyses.

We also have used this procedure to study the hydrolysis of D-glucufuranurono-6,3-lactone in hot (90°) water. By analyzing the reaction at timed intervals (Fig. 2), it is possible to determine directly the concentrations of the lactone (Peak 2), the D-glucuronic acid (Peak 1) produced by its hydrolysis, and to monitor the production of an unknown byproduct (Peak 3). In this manner, the rate of formation of D-glucuronic acid and the rate of disappearance of D-glucufuranurono-6,3-lactone as well as the final equilibrium concentrations of these two compounds may be ascertained directly (Fig. 3).

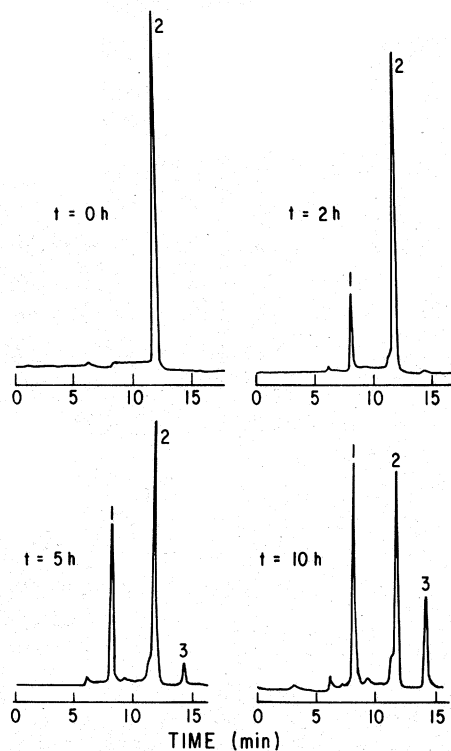


Fig. 2. Monitoring the hydrolysis of D-glucufuranurono-6,3-lactone (2) in aqueous solution at 90° by l.c. at various reaction times. Peak 1, D-glucuronic acid; Peak 3, unknown. Initial lactone concentration was 5 mg/mL. Other conditions, same as Fig. 1.

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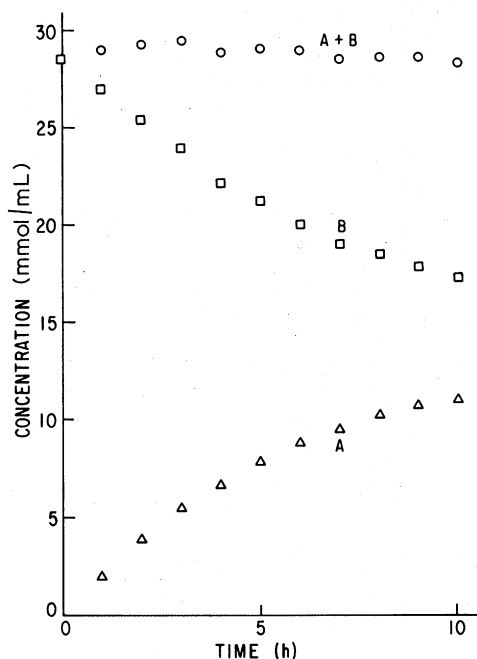


Fig. 3. Change in concentrations of D-glucuronic acid (A) and D-glucofuranurono-6,3-lactone (B) at various times in the reaction described in Fig. 2 as determined by l.c. The open circles represent the total concentration (mmol/mL) of both species (A + B) at the given reaction time.

It should be noted that some sugar acids and lactones do not chromatograph well on this system. Essentially, only those compounds stable under these chromatographic conditions ($\text{pH} = 2$, $<35^\circ$, for 15 min or less) give single, narrow peaks. We have found, however, that many sugar acids and lactones, such as the hexuronic acids and their 6,3-lactones, many aldonic acids and their 1,4-lactones, and "2-keto-" and "5-ketogluconic" acids all fit this requirement¹¹. The less-stable 1,5-lactones of the aldonic acids we tested, however, tend to hydrolyze in this system, and therefore produce chromatographic peaks indistinguishable from the parent aldonic acid. Still, as shown in Fig. 4, chromatography on HPX-87- H^+ resin can provide information concerning the less-stable lactones of aldonic acids. In this example, a sample of L-mannonic acid (sodium salt) was analyzed (Fig. 4a), and then treated by a procedure²¹ that quantitatively converts the acid into lactones. The sample was then re-analyzed (Fig. 4b) and found to give rise to a number of peaks. Peak 3, the peak for L-mannonic acid, represents unstable lactone forms (58% of the total sample, such as L-mannono-1,5-lactone) that were immediately hydrolyzed to the acid in this chromatographic system. Peak 4, L-mannono-1,4-lactone, was produced in 12% yield, and the remaining 30% of the starting material (Peaks 1 and 2) eluted before L-mannonic acid and after the solvent (Peak S), in a region where di- and tri-saccharides are eluted. Upon adding base to the mixture,

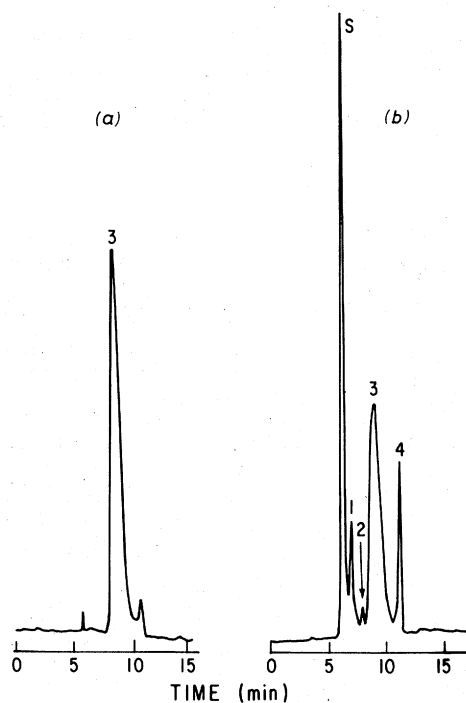


Fig. 4. Conversion of L-mannonic acid (3) (Fig. 4a) into stable lactone forms (Fig. 4b) as shown by l.c. For other peak assignments, see text. Chromatographic conditions as in Fig. 1 except that the initial L-mannonic acid concentration was 3 mg/mL.

Peaks 1, 2, and 4 disappeared, and the original amount of L-mannonic acid was produced. From these observations, the formation of intermolecular esters (Peaks 1 and 2) as well as unstable lactones is suspected.

In conclusion, the analysis of sugar acids and lactones by gel-permeation chromatography on cation-exchange resins is a convenient, rapid, and relatively high-resolution method that should be a useful alternative to the traditional anion exchange and gas-liquid chromatographic methods. It is especially useful for the simultaneous and direct analysis of stable sugar acids and lactones. Moreover, the columns used in this study were exceptionally stable (column life is 1 to 2 years), as they are continuously regenerated by the mineral acid in the mobile phase.

EXPERIMENTAL

Materials and methods. — All sugar acids and lactones were purchased from Sigma Chemical Company*. All chromatography was performed with a Dupont

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Model 8800 pump and heated column-compartment, a Rheodyne fixed-loop (20 μ L) injector, and a Gilson Holochrome u.v. detector (220 nm). Chromatograms were recorded on a Houston Instruments recorder. The column was an HPX-87-H⁺ model (Biorad Laboratories), containing spherical, 9- μ m diameter, sulfonated polystyrene-divinylbenzene strong-acid cation-exchange resin, that was eluted with degassed 5mM H₂SO₄. A precolumn containing HPX-85-H⁺ resin (Biorad Laboratories) was used in all cases. Standards were prepared fresh daily by dissolving pure sugar acids, their salts, or pure lactones in l.c.-grade water. Quantitation was accomplished by methodology with external standards as described elsewhere¹¹.

Saponification of D-mannofuranurono-6,3-lactone. — A stirred aqueous solution of lactone (6 mg/mL) was carefully titrated with an equivalent amount of M NaOH over the course of 30 min. Aliquots were withdrawn at intervals and injected directly into the l.c. system.

Reaction of D-glucofuranurono-6,3-lactone at elevated temperatures. — An aqueous solution of the lactone (5 mg/mL) was heated at 90° in a sealed flask. Aliquots were withdrawn at intervals and directly assayed by l.c.

Lactonization of L-mannonic acid. — The procedure of Lehrfeld²¹ was used. Thus, 75 mg L-mannonic acid (sodium salt) was converted into the free-acid form by passage through a column (7.5 mL) of IR-120-H⁺ resin. The effluent and a wash (30 mL of distilled water) of the column were evaporated to dryness at 45° in a rotary evaporator and then dried, *in vacuo*, for an additional 2 h at 85°. The resulting, amorphous solid was dissolved in 5 mL of H₂O and immediately analyzed by l.c. A portion of the product was saponified by adding M sodium hydroxide as already described and then assayed by l.c.

ACKNOWLEDGMENT

The author thanks S. Sondey for technical assistance.

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